How the litter-feeding bioturbator *Orchestia gammarellus* promotes late-successional saltmarsh vegetation

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Summary

1. Traditionally, studies on vegetation succession have focused either on plant–plant interactions or on interactions between plants and their physical environment, for example through organic matter build-up and increased nutrient cycling. These interactions can change conditions for macrodetritivores that feed on plant litter, but their role in vegetation succession is rarely studied. In this paper, we explore whether the bioturbating crustacean macrodetritivore *Orchestia gammarellus* alters soil conditions in a saltmarsh ecosystem in such a way that it promotes late-successional, less stress-tolerant plant species at the expense of early successional species.

2. To answer this, we performed a field and a laboratory experiment in which we manipulated abundances of *O. gammarellus*, and studied the consequences for soil physical and chemical parameters and for vegetation community composition.

3. Our field experiment showed that *O. gammarellus* stimulated nitrogen mineralization, likely resulting from the positive effect of this macrodetritivore on soil aeration and litter decomposition. Moreover, results from the laboratory experiment showed that *O. gammarellus* negatively affected dicot seedling survival of mainly early successional plant species, likely through grazing, thus affecting plant community composition.

4. The experiments together provided evidence that *O. gammarellus* promotes late-successional plant species in multiple ways: by alleviation of anoxic conditions, by promoting nutrient cycling and by selective herbivory on early successional species.

5. Synthesis. By demonstrating that a species traditionally considered as part of the detrital (‘brown’) food web is thus an important accelerator of vegetation succession, this study documents an important but often overlooked link in food web and ecosystem ecology.

Key-words: bioturbation, ecosystem engineering, macrodetritivores, N mineralization, plant–soil (below-ground) interactions, seedling predation, soil aeration, soil fauna, soil redox potential, vegetation succession

Introduction

Traditionally, vegetation succession is considered a plant-driven process (Clements 1916; Whittaker, Bush & Richards 1989; Glenn-Lewin, Peet & Veblen 1992; Tilman & Pacala 1993), where internal accumulation of nutrients in the ecosystem through litter production, with an associated increase in nutrient availability, is the most important driver of vegetation dynamics (Crocker & Major 1955; Bormann & Sidle 1990).

In addition, successional sequences often represent an abiotic stress gradient, with stress and environmental variability gradually decreasing towards later successional stages (Bazzaz 1979; Van Andel et al., 1993). There are several reasons why this abiotic stress gradient might occur. First, larger species in later successional stages (e.g. trees) may have a buffering effect on abiotic variability, thus reducing the abiotic extremes. Secondly, a more developed, organic-rich soil can buffer environmental extremes (such as drought events) better than very young soils, thus reducing abiotic stress levels (Crocker & Major 1955; Olson 1958; Adam 1990; Chapin II 1994).

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Macrodetrivores are known to interact strongly with the soil development process. In New Zealand and North America, for example, introductions of earthworms led to increased levels of soil aeration, litter fragmentation and nutrient cycling (Stock-dill 1982; Yeates 1991; Bohlen et al. 2004). In Scotland, diminished earthworm activity caused by an introduced predatory flatworm resulted in less aerated soils and a subsequent shift in vegetation composition (Boag & Yeates 2001). Examples of the impact of macrodetrivores on their environment extend beyond terrestrial ecosystems. In soft-bottom intertidal mudflats, lugworms (Arenicola marina) have been shown to bioturbate and promote oxygen diffusion in the upper 10 cm of the soil, which affects species composition and carbon and nitrogen cycling in the mudflat ecosystem (Volkenborn, Robertson & Reise 2009). By influencing the process of soil development—particularly in ecosystems that are regulated by abiotic stress—macrodetrivores may be of considerable importance for the dynamics of vegetation succession. However, there is a lack of experimental studies examining this potentially important interaction in the context of ecological succession.

Saltmarshes are an example of a strongly stress-dominated ecosystem (Davy et al. 2011), where soil anoxia and salinity present the two most important abiotic stress factors (Bertness & Leonard 1997). Traditionally, build-up of nutrients through sedimentation and litter production has been hypothesized to drive vegetation succession in this ecosystem (Olf et al. 1997; Van Wijnen 1999). Vegetation succession may then represent a shift from good colonizers and/or nutrient competitors to good light competitors with increasing soil fertility (Olf et al. 1997). On the other hand, experiments have shown that low nutrient availability is not the only factor preventing late-successional saltmarsh plants from establishing in abiotically stressful, early successional stages (Kuijper, Nijhoff & Bakker 2004), or in poorly aerated conditions (Kockelmann & Neuhaus 1999; Bockelmann et al. 2002; Davy et al. 2011; Mossman et al. 2012). These studies suggest that late-successional saltmarsh plants are possibly poorly adapted to the adverse soil conditions (i.e. high salinity and low oxygen availability) present in early successional stages, which require specific adaptations (such as salt excretion strategies and high root aerenchyma; Justin & Armstrong 1987; Vartapetian & Jackson 1997; Bailey-Serres & Voesenek 2008). This idea is supported by recent studies, which illustrate that late-successional saltmarsh species are clearly restricted to well-aerated conditions in late succession (Davy et al. 2011; Mossman et al. 2012). Although seemingly essential for vegetation succession, exactly how these more benign growing conditions for plants might arise remains largely unresolved.

Activity of burrowing macrodetrivores on saltmarshes could potentially result in amelioration of stress and, hence, more beneficial soil conditions for late-successional plant species, thus promoting their competitive strength compared to early successional plant species. On European saltmarshes, however, bioturbating earthworms (a dominant macrodetrivore in most terrestrial ecosystems) are completely lacking, likely due to their low tolerance to salinity (Piearce & Piearce 1979). Also burrowing crab species, which have been reported to reduce soil anoxia on American saltmarshes, are absent from these marshes (Bertness 1985; Daleo et al. 2007). Instead, the dominant macrodetrivore present is a small (0.5–1.5 cm) crustacean, the amphipod Orchestia gammarellus (Pallas 1776; henceforth O. gammarellus). It occurs in very high abundances on saltmarshes with a low to intermediate elevation where inundations with salt water are most frequent (Moore & Francis 1986; Meyer et al. 1995; Dias & Sprung 2003) in the same area where anoxia stress to plants is most pronounced. It can occur in densities up to 3500 individuals per square metre (hereafter ind. m\(^2\)), but typically occurs in densities around 500–700 ind. m\(^{-2}\) (Dias & Sprung 2003; Laffaille et al. 2005). Orchestia gammarellus has been shown to exhibit extensive bioturbation activity where it digs shallow tunnels in the topsoil, presumably to avoid temporary desiccation stress and to access decomposing plant litter (Moore & Francis 1986; Dias & Sprung 2003). Moreover, as a litter fragmenter it can affect leaf litter decomposition rates (Hemminga & Buth 1991) and can therefore be expected to impact on N cycling. Similar to related amphipods, it likely does not feed on the litter itself, but on the attached microorganisms found on the litter fragments that it ingests, which can promote further microbial decomposition of the litter and mineralization of nutrients (Fenchel 1970; Lopez, Levinton & Slobodkin 1977).

In order to understand whether the abundance of O. gammarellus can change the competitive balance between early and late-successional saltmarsh plant species, we address the following questions: (i) What is the influence of O. gammarellus on soil aeration, the rate of litter decomposition and N mineralization, and (ii) to what extent does this result in changes in plant species composition and productivity? We expect that promotion of soil aeration and the facilitation of litter decomposition by O. gammarellus result in enhanced nitrogen mineralization, which are further expected to result in a higher availability of nitrogen to plants. Ultimately, we predict these soil physical changes to lead to vegetation change, such that late-successional species are promoted at the expense of early successional species.

To test these predictions, we performed field measurements on abundances of O. gammarellus and related these to local environmental conditions. To study the effects of O. gammarellus on various biotic and abiotic parameters, most notably soil anoxia, soil net nitrogen mineralization and vegetation composition and productivity, we conducted a laboratory and a field experiment where we manipulated abundances of O. gammarellus.

Materials and methods

STUDY SITE

We conducted field measurements of O. gammarellus abundances and relevant biotic and abiotic at the saltmarsh of the coastal barrier island of Schiermonnikoog, the Netherlands (53°30' N, 6°10' E).
Three plots were spaced at least 100 m apart at each of six sampling locations that were spread evenly along an 8 km gradient of vegetation succession (see Fig. S1 in Supporting Information; see Schrama, Olff & Berg 2012 for a full description of site characteristics). Along this gradient, vegetation composition changes from a species-rich halophytic plant species community on a periodically anoxic clay soil in early succession (dominant plant species: Limonium vulgare and Puccinellia maritima) towards a mixed stand of marginal halophytic species on a well-aerated clay soil in late succession (dominant plant species: Eltigia atherica and Festuca rubra). To maintain a constant inundation frequency, all plots were located at an average elevation of 10 cm (±SD 7.7 cm) above mean high tide.

To study the effects of varying abundances of O. gammarellus on biotic and abiotic parameters in early and late-successional vegetation types, we conducted field experiments on a part of the saltmarsh where vegetation succession started on intertidal sand flats in 1967 (Olff et al. 1997).

Our mesocosm experiment was designed to study biotic and abiotic effects of O. gammarellus on plants under controlled conditions. Soil samples for this experiment were taken from a 120-year-old part of the saltmarsh (Olff et al. 1997), which is grazed by cattle at a stocking density of 0.5 cows ha$^{-1}$ (Ros et al. 2002). Orchestia gammarellus and E. atherica did not occur in these samples. Specimens from both species were collected at an adjacent ungrazed part of the saltmarsh, located 500 m eastward.

**FIELD MEASUREMENTS: THE RELATIONSHIP BETWEEN O. GAMMARELLUS AND RELEVANT ENVIRONMENTAL FACTORS**

We sampled densities and relevant environmental variables across all 18 plots. Orchestia gammarellus was sampled in October 2008. Environmental variables were measured between October 2008 and November 2009. Quantitative estimations of abundances were made using enclosed pitfall traps (each location with one Ø 10 cm trap, in the SW corner of 50 × 50 cm Perspex enclosures, after Lang (2000)), filled with glycerol to preserve specimens. Pitfalls were emptied every 5 days over a period of 18 days, until no more O. gammarellus individuals were found in the traps.

As a proxy for soil aeration, we measured soil redox potential at each sampling site, using platinum electrodes (Pt) and one AgCl-reference electrode (Cole-Palmer, Vernon Hills, IL, USA). These electrodes were connected to a Datalogger (type GL200; Graphitec, Tokyo, Japan) and placed in a square design (±25 times 25 cm), 10 cm from the reference electrode, at a soil depth of 2 cm. Redox potential values were read out two minutes after the electrodes were inserted to standardize measurements. Averages of the four Pt-electrodes were corrected for the value of the AgCl-reference electrode (+197 mV). Although this measurement does not provide absolute values of the amount of oxygen in situ, it gives a reasonable proxy for the relative differences in soil aeration between locations measured on the same day (Van Bochove, Beauchemin & Theriault 2002).

We determined total above-ground plant peak biomass (including standing dead and litter) by clipping a 50 × 50 cm square at 1 cm above the soil surface, which was dried in a stove at 70 °C for 48 h. The percentage cover for every plant species at each of the sampling points was estimated using the Londo cover method (Londo 1976) in July 2009.

As the thickness of the accumulated layer of clay is a good proxy for the total soil N pool (Olff et al. 1997), we determined sediment layer thickness on top of the sandy base layer with a sediment core (Ø 1 cm), from which layer thickness was estimated to the nearest millimetre.

**TESTING THE EFFECT OF O. GAMMARELLUS ON NUTRIENT CYCLING AND VEGETATION PRODUCTIVITY**

To investigate the hypothesis that O. gammarellus speeds up nutrient cycling, modifies soil physical properties and changes vegetation composition, we carried out a field experiment between November 2008 and July 2010 in which we manipulated the abundance of O. gammarellus. Treatments were placed into an early successional mixed L. vulgare and P. maritima vegetation in November 2008 and in a late-successional E. atherica-dominated vegetation type in July 2009. Enclosures consisted of 1 × 1 m mesh fence (1 mm mesh size), which was dug into the soil to a depth of 25 cm. Each site contained three enclosed treatments: O. gammarellus addition (henceforth addition treatment), O. gammarellus removal (henceforth: removal treatment) and a control treatment. Triplets of enclosures were replicated in both vegetation types using a randomized block design with five blocks spaced at least 50 m apart. Every other month, 5-day manipulations of O. gammarellus densities were carried out using pit-falls in the south-west corner in each of the enclosures, as monthly springtides were expected to cause redistribution of O. gammarellus within the blocks. All O. gammarellus from the removal treatment were counted and transported to the addition treatment. The O. gammarellus from the control and addition treatments were caught, counted and released again in their own enclosure. In general, experimental manipulations were successful (see Fig. S2).

To investigate the effect of O. gammarellus abundance on leaf litter decomposition, litterbags were placed in each of the five replicate blocks between 13 December 2008 and 3 March 2009 (12 weeks), in both vegetation types. This time period was chosen because most litter is produced in autumn and gets decomposed over the course of winter and early spring (Hemminga, Kok & De Munck 1988), as temperatures are not too low for decomposition in the maritime climate on this island. Orchestia gammarellus is generally active at temperatures above zero degrees (Moore & Francis 1986). Litterbags (5 × 12 cm, mesh size 1.5 mm) excluded O. gammarellus, but allowed entrance of small-sized decomposers. We added another set of litterbags with similar mesh size, but with 20 additional holes (Ø 5 mm), allowing O. gammarellus to enter (Buth & De Wolf 1985). Litterbags were filled with 10 g of dried, fragmented E. atherica leaf litter (fragment size ±2 cm, dried at 70 °C, for 48 h). After the field incubation, litterbags were brought back to the laboratory, where clay and sand were carefully rinsed off on a sieve (mesh size 250 μm) after which the litter was dried (70 °C, 48 h), and weighed to the nearest 0.01 g.

Nitrogen mineralization was measured in situ using mineralization tubes (Berendse, Lambers & Olff 1998; Van Wijnen, van der Wal & Bakker 1999) between 2 November 2008 and 16 July 2010. PVC tubes (Ø 5 cm; 14 cm high) were drilled in the upper 10 cm of the soil and were sealed off with a plastic lid to prevent plant in-growth. Water exchange was allowed via small holes (Ø 0.5 cm) at soil level. Nitrogen-free Miracloth® (EMD Millipore, San Diego, CA, USA) was put on the bottom of the tubes to allow water levels to rise and drop inside the tube as well as to prevent in-growth of roots. In each treatment, we collected one reference tube and placed an incubation tube right next to it at the start of each incubation period. This procedure was continued throughout the experiment. The reference samples were directly transported to the laboratory and were handled the same way as the field-incubated samples. Soil was removed from the tubes.
and all fresh roots were carefully removed, after which the samples were well mixed by hand and 25 g of fresh soil was taken for analysis. All plant-available NO$_3^-$ and NH$_4^+$ were dissolved into 60 mL of 1 M KCl and were well mixed for 16 h at 60 rpm using a custom-made rotating shaker. Afterwards, samples were filtered over a nitrogen-free filter paper and analysed for NO$_3^-$ and NH$_4^+$ using an auto-analysing (Navone 1964; Searle 1984; Type 5100; Skalar BV, Breda, the Netherlands).

IMPACT OF O. GAMMARELLUS ON SOIL AERATION AND VEGETATION COMPOSITION

We performed a mesocosm laboratory experiment to study the effect of O. gammarellus on growing conditions of E. atherica. Twenty intact, compacted clay soils with no O. gammarellus and E. atherica were collected from the field using PVC rings with a sharp edge (Ø 25 cm, 8 cm height). We added 100 individuals of O. gammarellus to half of these PVC rings (n = 10), which resembles an abundance of 2000 ind. m$^{-2}$. Every ring was fenced with a 25-cm-high transparent plastic sheet to prevent O. gammarellus from escaping. To mimic environmental conditions of the lower saltmarsh, rings were placed in a thin (2-3 mm) layer of artificial sea water (34 g InstantOcean® L$^{-1}$ diluted in 1 L demineralized water), which was replaced every 2 weeks to avoid algal growth. The experiment was performed in a climate chamber, set at 70% RH. Temperature was set at 17 degrees during the day (6 am – 8 pm) and 14 degrees at night (8 pm – 6 am). Four E. atherica individuals were planted in each mesocosm. Each of these plants was grown from a 2-cm rhizome for 21 days. The mean initial wet weight of the planted E. atherica individuals was 13.7 g (±SE 1.2 g) in mesocosms without O. gammarellus and 12.4 g (±SE 0.6 g) with O. gammarellus. To mimic rainfall, all mesocosms were sprayed with 10 mL fresh water every 3-4 days, using a hand-held sprayer. Capillary action further prevented drying out of the cores.

To quantify the effects of O. gammarellus treatments on leaf litter decomposition, we added 10 g (±SE 0.1 g) of dried (70°C, for 48 h) E. atherica litter at the start of the experiment, 1 day before O. gammarellus was put in, and another 10 g was added after 4 weeks. At the end of the experiment, the remaining litter was removed using forceps, rinsed in a 300-μm sieve to get rid of clay particles, dried (70°C, 48 h) and weighed to determine mass loss.

In order to estimate the effects of O. gammarellus on soil aeration, we measured soil redox potential (see above) inside the mesocosms biweekly, as a proxy for the level of oxygen in the soil. All four measurement electrodes were first inserted at 1 cm depth and subsequently at 4 cm depth. Values for each depth were read out after 2 min. At the end of the experiment, the number of newly formed shoots on every individual of E. atherica was counted. Live above-ground mass was clipped at soil level and was subsequently dried (70°C, 48 h). The remaining number of living O. gammarellus individuals was counted by careful inspection of all mesocosms by hand. The effects of exclusion of O. gammarellus on seedlings that emerged from the soil seed bank were investigated by identifying, counting and weighing the number of emerged seedlings for each plant species.

STATISTICAL ANALYSES

To investigate the relationship between the abundance of O. gammarellus and the different environmental factors in the field, we used pairwise Pearson’s correlations. To detect the most important predictors for the presence of O. gammarellus, we used a generalized linear regression model on logarithmically transformed O. gammarellus abundances with a log link function. The full models consisted of all main effects. Non-significant effects (P > 0.05) were removed from the models in a backwards stepwise fashion, starting with non-significant interaction terms.

Nutrient mineralization was analysed using a GLMM with treatment (three levels: control and O. gammarellus addition or removal) and replicate as categorical predictors. Pairwise t-tests were used to compare treatments with O. gammarellus addition and removal. Non-normally distributed dependent variables were tested between the two treatments using a nonparametric Mann–Whitney U-test. Results from the mesocosm experiment were tested with a GLMM, with treatment (two levels: with or without O. gammarellus) as a fixed factor and position in the climate chamber as a random factor (blocks). A GLMM with treatment (two levels: with or without O. gammarellus) and depth (two levels: 2 and 4 cm in the soil) as categorical predictors was used to test the differences between soil redox potentials in mesocosms with and without O. gammarellus, where the average of the four electrodes was used as a response variable. All statistics were done using stata® 9.0 (Statasoft, Tulsa, OK, USA).

RESULTS

RELATIONSHIP BETWEEN ABUNDANCES OF O. GAMMARELLUS AND ENVIRONMENTAL VARIABLES

Orchestia gammarellus abundances varied between 68 ind. m$^{-2}$ in the earliest successional vegetation type and 3492 ind. m$^{-2}$ at the latest vegetation type (average 1002 ind. m$^{-2}$) and were positively correlated with soil redox potential ($R^2 = 0.57$, $P < 0.001$), standing dead biomass ($R^2 = 0.28$, $P = 0.02$), thickness of clay layer ($R^2 = 0.52$, $P < 0.001$) and vegetation height ($R^2 = 0.48$, $P = 0.001$). Abundances were negatively correlated with soil moisture content ($R^2 = 0.51$, $P < 0.01$) and were uncorrelated with total plant biomass.

Results from the generalized multiple regression analysis showed that soil redox potential explained most variation in the spatial distribution of O. gammarellus across the successive gradient (Wald Stat = 24.41; $P < 0.001$, see Table S2). Abundances of O. gammarellus increased with soil redox potential values (soil redox potential > 50 mV, Fig. 1a), indicating that higher abundances were associated with dryer and more aerobic conditions. A linear correlation between the abundance of O. gammarellus and soil redox potential was significant ($R^2 = 0.54$, $F_{1,16} = 21.4$, $P < 0.001$), but a quadratic fit gave a higher coefficient of determination ($R^2 = 0.67$, $F_{1,15} = 18.1$, $P < 0.001$; Fig. 1a). In turn, soil redox potential values correlated strongly with soil moisture ($R^2 = 0.84$, $F_{1,15} = 45.1$, $P < 0.001$; Fig. 1b). Soils were found to be highly anoxic (≈0 mV) when soil moisture values were above 54% of the total soil volume.

RESULTS: FIELD MANIPULATIONS

Effects on litter decomposition

A few small, juvenile O. gammarellus were found in the small mesh size litterbags, indicating that litterbags were not
fully closed to *O. gammarellus*. In the early successional vegetation type, in the presence of *O. gammarellus*, 68% and 72% of litter mass loss occurred in the control and addition treatments, respectively, while only 59% mass loss was recorded in the removal treatment (*F*<sub>2,4</sub> = 3.6, *P* < 0.05, Fig. 2a). Litter decomposition was on average 10% lower in litterbags closed off to *O. gammarellus* in the early vegetation type (*F*<sub>1,20</sub> = 4.4, *P* = 0.02). Although trends were similar, decomposition in late-successional vegetation type did not differ significantly between addition, control and removal treatments (Fig. 2b).

**Effect on N mineralization in the field**

Removal of *O. gammarellus* reduced N mineralization in both early and late-successional vegetation types, but only in summer when the peak in mineralization occurs (Fig. 3a). In early successional vegetation types, we found a 32% and 43% lower total N mineralization in the removal treatment in June 2009 and July 2010, respectively. Similarly, in the late-successional vegetation type, we found a 42% lower total N mineralization in the removal treatment in July 2010 (Fig. 3b). The total N mineralization in the addition treatment was also significantly higher than in the removal treatment (*P* < 0.05), but was not different from the control treatment, indicating that removal rather than addition of *O. gammarellus* resulted in changes in N mineralization. Mineralization of NH<sub>4</sub>-N was similar between treatments, whereas NO<sub>3</sub>-N production was higher for the addition treatments, suggesting in combination with the redox results that soil aeration due to bioturbation behaviour of *O. gammarellus* promoted not only total N mineralization but also nitrification rates (Fig. 3b).

**Effects on peak above-ground standing plant biomass**

We found an overall positive effect of *O. gammarellus* addition on above-ground peak standing vegetation mass (*F*<sub>2,18</sub> = 7.0, *P* = 0.006; Fig. 4). Addition of *O. gammarellus* resulted in a significantly higher above-ground peak standing plant biomass in the early successional vegetation type, when compared to the removal treatment (*t* = 6.8, *n* = 5, *P* = 0.003). The pattern was similar for the late-successional vegetation type, but differences between removal and addition treatments were only marginally significant (*t* = 2.9, *n* = 4, *P* = 0.06; Fig. 4).

**RESULTS: MESOCOSM MANIPULATIONS**

**Effect of addition of *O. gammarellus* on seedling establishment in the mesocosm experiment**

On average, 72% of all *O. gammarellus* survived (range: 62–84 of the initial 100 ind.) until the end of the experiment,

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*Fig. 1.* Relationship between soil redox potential, numbers of *Orchestia gammarellus* and soil moisture. (a) Relationship between soil redox and numbers of *O. gammarellus*. (b) Relationship between soil moisture and numbers of *O. gammarellus*. Size of dots corresponds to soil moisture level (panel a) and abundance of *O. gammarellus* (panel b).

*Fig. 2.* Litter decomposition in litterbags (*n* = 5) with different mesh sizes, incubated in *O. gammarellus* removal (black), control (dark grey) and *O. gammarellus* addition plots (light grey) in early successional vegetation (a) and late-successional vegetation (b). Litter removal was determined after 12 weeks of incubation. Bars denote averages (±SE) of litter bags. Letters denote significant differences between treatments at *α* < 0.05.
with some variation between pots. In 8 out of 10 mesocosms where *O. gammarellus* was absent, we found clear signs of fungal growth, whereas in the mesocosms with *O. gammarellus*, no fungal growth was observed.

Although no plant seeds were added, spontaneous recruitment from the soil seed bank occurred. In the absence of *O. gammarellus*, we found a greater number of established seedlings (*O. gammarellus* absent: 24.7/6.7 seedlings vs. *O. gammarellus* present: 0.7/0.3 seedlings; \(t = 3.8, n = 10, P < 0.002\)), as well as a higher seedling total mass (*O. gammarellus* absent: 0.76 g/0.15 vs. *O. gammarellus* present: 0.03 g/0.02; \(t = 5.0, n = 10, P < 0.001\)). The majority of the seedlings in the absence of *O. gammarellus* belonged to *Salicornia* spp. (68% of all seedlings) and *Suaeda maritima* (12%) and only a small fraction belonged to *E. atherica* (2.4%). In the presence of *O. gammarellus*, the majority of the seedlings belonged to *E. atherica* (57% of all seedlings). The absolute number of *E. atherica* seedlings was not different between treatments. Furthermore, we found no significant correlation between seedling mass and the number of surviving *O. gammarellus* (\(P > 0.5\)).

**Effect on E. atherica** biomass production in the mesocosms

Similar numbers of *E. atherica* plants died in the presence and absence of *O. gammarellus* in the first week of the experiment (20% and 17.5%, respectively). These plants were excluded from further comparisons between treatment effects on plants. No plants died in the remaining 7 weeks of the experiment.

Overall, we found a 40% higher weight of *E. atherica* per ramet tiller, in the presence of *O. gammarellus* (Table 1). The number of newly formed tillers was not affected by the presence or absence of *O. gammarellus* (Table 1). Weight of above-ground standing dead mass was 58% lower in the *O. gammarellus* addition treatment, which was most likely due to consumption of the dead plant parts by *O. gammarellus* (Table 1).

**Effect of O. gammarellus** on soil redox potential in the mesocosms

Initial measurements in all mesocosms showed negative soil redox potential values at the start of the experiment, indicat-
Table 1. Soil physical properties and vegetation variables in response to the presence and absence of Orchestia gammarellus in the mesocosm experiment after 8 weeks

<table>
<thead>
<tr>
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<th>Without O. gammarellus</th>
<th>With O. gammarellus</th>
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<tr>
<td><strong>Mean</strong></td>
<td><strong>Mean</strong></td>
<td><strong>Mean</strong></td>
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<tr>
<td>Soil moisture (%)</td>
<td>34.55 0.78</td>
<td>32.09 0.48</td>
</tr>
<tr>
<td>Total number of Elytrigia atherica tillers</td>
<td>2.23 0.38</td>
<td>3.03 0.43</td>
</tr>
<tr>
<td>Number of newly formed E. atherica tillers</td>
<td>0.63 0.18</td>
<td>1.37 0.32</td>
</tr>
<tr>
<td>Dry weight of live above-ground parts of E. atherica (g. ind⁻¹)</td>
<td>0.18 0.04</td>
<td>0.25 0.03</td>
</tr>
<tr>
<td>Dry weight of dead above-ground parts of E. atherica (g. ind⁻¹)</td>
<td>0.26 0.04</td>
<td>0.11 0.02</td>
</tr>
<tr>
<td>Percentage of litter decomposed</td>
<td>31.75 1.23</td>
<td>43.2 0.88</td>
</tr>
<tr>
<td>Soil extractable NH₄⁺ (g kg⁻¹)</td>
<td>7.97 0.74</td>
<td>8.06 0.74</td>
</tr>
<tr>
<td>Soil extractable NO₃⁻ (g kg⁻¹)</td>
<td>0.49 0.08</td>
<td>1.32 0.19</td>
</tr>
<tr>
<td>Soil extractable total mineral N (g kg⁻¹)</td>
<td>8.54 0.7</td>
<td>9.27 0.8</td>
</tr>
<tr>
<td>Total biomass of seedlings (g per mesocosm)</td>
<td>0.76 0.15</td>
<td>0.03 0.02</td>
</tr>
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Bold P-values indicate significant effects.

**Effect of O. gammarellus on decomposition and nitrogen availability in the mesocosms**

In general, litter decomposed significantly faster in the presence of O. gammarellus; 41% was decomposed in the O. gammarellus addition treatment after 8 weeks, while 30% of the litter decomposed in the absence of O. gammarellus (Table 1).

Total extractable NO₃⁻ was 164% higher in the presence of O. gammarellus (t-test, t = 4.10; n = 10; P < 0.001), but there was no significant effect on the availability of NH₄⁺ (Table 1). The ratio of available NO₃⁻ : NH₄⁺ was higher in the O. gammarellus addition treatment (t-test, t = 3.11; n = 10; P = 0.006), indicating higher nitrification. We found a positive relationship between the rate of decomposition and the extractable NO₃⁻ levels (R² = 0.38, n = 10; P = 0.004), supporting the notion that higher NO₃⁻ production is the result of higher activity of O. gammarellus.

**Discussion**

This study shows that O. gammarellus can impact on saltmarsh vegetation succession by acting on a number of key ecosystem processes that directly or indirectly relate to soil aeration. We argue that this may add to understanding previous studies, where late-successional grass species could not establish in low-elevation saltmarshes or anoxic conditions and early successional stages (Kuijper, Dubbeld & Bakker 2005; Mossman et al. 2012), even in the absence of competition for nutrients or grazing.

**Effects of O. Gammarellus on the Abiotic Stress Landscape of the Saltmarsh**

We expected that bioturbating behaviour by O. gammarellus would promote soil conditions that are beneficial for plant growth. Indeed, evidence from both our mesocosm and field experiment indicates that the presence of O. gammarellus enhances soil aeration, leaf litter decomposition and N mineralization. Earlier studies have already pointed out the potential importance of the digging and burrowing behaviour of this species (Moore & Francis 1986; Dias & Sprung 2003), but none of these studies has related this behaviour to changes in soil physical, chemical and biological properties, and to vegetation change. Increasingly, redox potential is being shown to

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Fig. 5. Soil redox potential (±SE) over time at a depth of 1 cm (a) and 4 cm (b), in treatments with and without Orchestia gammarellus. *P < 0.05, **P < 0.01, ***P < 0.001.
be an important factor underlying distribution of vegetation on saltmarshes (Davy et al. 2011; Mossman et al. 2012). Our field measurements show that high abundance of O. gammarellus correlates strongly with a higher soil redox potential in later successional vegetation stages. Moore & Francis (1986) pointed out that O. gammarellus could be sensitive to low oxygen levels and high redox values, while Diptera larvae – which are more abundant in early succession (Schrama, Olff & Berg 2012) – are perhaps better able to cope with these circumstances. Diptera, however, lack the ‘ecosystem engineering effect’ of creating an oxic top soil layer that results from burrowing. We therefore confirm our hypothesis that bioturbation is an important behavioural characteristic of O. gammarellus which promotes its own presence and impacts the abiotic stress landscape on the saltmarsh.

**EFFECT ON NUTRIENT MINERALIZATION AND VEGETATION PRODUCTION**

Positive effects of other macrodetritivores on vegetation productivity have been observed in a great number of ecosystems, which is often attributed to their positive influence on N mineralization by leaf litter fragmentation, litter assimilation and stimulation of microbial activity and increase in biomass (Bardgett & Wardle 2010). Our visual observations on the lack of fungi on litter in the presence of O. gammarellus also suggest that it is likely that O. gammarellus does not engage actively in decomposition of litter, but rather speeds up litter decomposition by micro-organisms. We therefore suggest that positive effects of O. gammarellus on N mineralization occur predominantly via litter fragmentation (increasing the surface area for microbes) and inoculation of fresh litter with microbes which positively affects microbial litter decomposition (Hanlon & Anderson 1980; Scheu & Wolters 1991; Heemsbergen et al. 2004). The observed higher production of NO$_3^-$ in the presence of O. gammarellus can only occur when oxygen is abundant (Laanbroek 1990), thus suggesting that, additionally, part of the positive effect operates via soil aeration. Another explanation for the reported positive effect of O. gammarellus on N mineralization is that addition of O. gammarellus itself may have resulted in higher N mineralization. Assuming that all O. gammarellus died after addition to the field enclosures, and given a N content of 20% in their tissue, only 0.03 g N m$^{-2}$ would have been added between April and June 2009, while the observed difference in N mineralization was 1.22 g N m$^{-2}$ (for details on calculation, see Table S1). We therefore conclude that the addition of O. gammarellus followed by mortality cannot solely account for the strong increase in N mineralization in the addition treatment.

Vegetation on a temperate back barrier saltmarsh is generally N-limited (Ollf et al. 1997; Van Wijnen & Bakker 1999; Kuijper, Dubbeld & Bakker 2005). Hence, increased availability of N is likely to result in higher vegetation productivity. Results from our field experiment showed that high O. gammarellus abundance increased peak biomass and N mineralization, particularly in spring and summer measurements, when vegetation is most productive. This implies that O. gammarellus can alter soil physical conditions and vegetation productivity, ultimately impacting on successional dynamics.

**EFFECTS ON PLANT COMMUNITY COMPOSITION**

The effect of O. gammarellus on vegetation composition may partly be due to its observed impact on dicot seedling survival. Orchestia gammarellus is generally characterized as a macrodetritivore species (Moore & Francis 1986), although predation on living plant tissues may be a way in which macrodetritivores support their diet with N (Griffith et al. 2013). Interestingly, the majority of the surviving seedlings in the treatment with added O. gammarellus belonged to E. atherica. Studies by Brown & Gange (1992), Hulme (1994) and Allan & Crawley (2011) on seedling predation by snails all mention a more negative impact on dicots than on graminoids, which could be due to the general lower palatability of
grasses. This strengthens our notion that *O. gammarellus* – which is often found in the litter layer of dense stands of *E. atherica* – has a relatively positive influence on (late-successional) grass species and a relatively negative influence on emerging forbs. We suggest that this selective foraging on living tissue is motivated by plant resource quality. Although no conclusive data on quality differences between seedlings exist, earlier studies have shown that the late-successional grass *E. atherica* is not preferred by geese (Van der Wal et al. 2000) and hare (Kuijper, Nijhoff & Bakker 2004), as the lignin content of this species is much higher than that of other species on the saltmarsh. High lignin content is also known to reduce litter palatability for other macrodetritivores, such as millipedes and isopods (Cotrufo, Briones & Ineson 1998). Thus, we conclude that seedling predation by *O. gammarellus* could impact on the plant community composition by (indirectly) favouring lower quality, late-successional grasses. Combined with recent observations that earthworms can actively graze on living plants (Griffith et al. 2013), this suggests that the role of macrodetritivores as herbivores in plant communities may be greater than previously thought.

In conclusion, we found strong indications that the macro-detritivore *O. gammarellus* engages in nutrient dynamics, soil aeration and seedling predation on saltmarshes, all of which favour the late-successional grass species *E. atherica* above early successional species. As such, *O. gammarellus* not only affects its own presence positively, but also may play a key role in structuring soils and vegetation communities on low and middle saltmarshes in western Europe (Fig. 6). Where early successional stages on middle and low European saltmarshes are dominated by small vertebrate herbivores (hare and geese; Fig. 6a), late-successional stages become progressively dominated by detritivores such as *O. gammarellus* (Fig. 6b). This transformation coincides with important changes in soil physical, chemical and biological factors, all of which have been shown here to be affected to some extend by *O. gammarellus*. Historically, mechanisms explaining vegetation succession focused on plants and their interactions with other plants and with the physical environment (Clements 1916; Bormann & Sidle 1990). More recently, plant pathogens have been identified as important drivers of vegetation succession (Van der Putten 2003; Kardol, Bezemter & Van der Putten 2006). When aiming at understanding the drivers that underlie ecological succession, our results indicate that other parts of the below-ground food web such as macro-detritivores may also be an important driver. By demonstrating that a species traditionally considered as part of the detrital food web can alleviate abiotic stress and modify the plant community composition by seedling herbivory, this study documents an important but often overlooked link in food web ecology.

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**Data accessibility**

Data are available at doi:10.5061/dryad.6k72n.

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